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Biomarkers in NSCLC Epidermal Growth Factor Receptor Mutations

Suzane Freidman

Abstract

Lung Cancer is the most common global cause of cancer related deaths in men and women (Markus, Alain, 2013). As standard radiation and chemotherapy have proved ineffective, novel target therapies are in the midst of development. This review will analyze the success of the inhibitor drugs targeting the Epidermal Growth Factor Receptor (EGFR) mutation, commonly found amongst Lung Cancer patients. Numerous studies and reviews are utilized to determine the cause of the 10% success rate currently exhibited for these drugs. The L858R and E746-A750 point mutations and deletions respectively, were found prevalent in responsive patients as well as clinical-pathological features such as female gender, Asian descent, non-smoking history, and Adenocarcinoma. Adenocarcinoma was found almost exclusively in responsive patients and non-smoking history is proposed to have an independent correlation to EGFR mutations (Kosaka, et.al. 2004). These prevailing features can be used as biomarkers to predict the responsiveness of a patient population, leading to efficient and successful distribution of the EGFR inhibition drug to Lung Cancer patients.

Introduction

The Epidermal Growth Factor Receptor (EGFR), also known as ErbB1 or Her1 in humans, was first discovered by Stanley Cohen in the 1970's (Yarden, Sliwkowski, 2001). Since then, our understanding of the protein and its critical role in numerous forms of cancer has advanced in enormous strides. Advances in this area have proven to be fruitful, and much hope is put into future development for cancer cures. EGFR is now understood to be one of four members of the ErbB interactive family of Tyrosine Kinase (TK) Proteins. EGFR, as well as ErbB 2, 3, and 4 (Her 2, 3, 4) are phosphorylation inducing proteins on the Tyrosine portion of the receptors (Yarden, Sliwkowski, 2001). EGFR carries out numerous signal transduction pathways which result in cellular proliferation, metastasis, and apoptotic aversion, all clearly oncogenic activities. Consequently, EGFR commonly causes numerous types of cancers whenever-expressed in epithelial tissue throughout the body (Heist, Christiani, 2009). In Non Small Cell Lung Cancer (NSCLC) specifically, EGFR over expression is the most common cause, leading to much research and analysis of this protein, and its mutations in regard to lung cancer. Numerous drug therapies have been developed to target the specific mutations that were found as causes of NSCLC. Understanding of the structure and mechanism of

this protein is crucial to the development of biomarkers to target specific populations containing the mutation to allow for further development of target therapy for cancer.

Methods

Various databases were utilized to gather papers on research studies and reviews on the subject matter to analyze it and determine possible biomarker methods and their efficiency. Articles were found in Google Scholar, Touro Library Database, and Rutgers University Database. These papers were analyzed and used to come to the conclusions explained in the discussion of this paper.

Results:

EGFR

EGFR is a tri-domain membranous receptor protein consisting of an extracellular ligand-binding domain mainly comprised of cysteine, a hydrophobic transmembranous area, and an intracellular Tyrosine Kinase Domain (Yarden, 2001). The Tyrosine Kinase domain is made up of a juxtamembrane region, an area for TK activity, and a C-terminal domain (Markus, Alain, 2013). A number of ligands contain an affinity to EGFR including the Transforming Growth Factor- α (TGF- α), Epidermal Growth Factor (EGF) and Amphiregulin (Figure 1) (Huang, Harari, 1999). All EGFR ligands contain around 50 amino acids including six

cysteine residues in β -sheet formation and are highly regulated by numerous signal transduction pathways throughout the cell (Yarden, Sliwkowski, 2001). TGF- α is the most common ligand (Vealel, et. al. 1987). Following EGFR binding, dimerization of EGFR occurs to form a homodimer with an identical receptor or to form a heterodimer with a different protein of the ErbB family (Isobe et. al. 2005). Heterodimers are stronger than homodimers with a smaller rate of ligand dissociation, increased period of tie at the cell surface, and increased mitogenic capabilities. Dimerization leads to autophosphorylation of Tyrosine residues of the cytoplasmic region of the protein itself. The specific regions activated on the Kinase domain, and consequently the specific pathways activated, are dependent on the ligand's identity as well as EGFR's dimerization partner. EGFR's activation allows it to bind to cytoplasmic messenger proteins which initiate various signal transduction cascades resulting in cell proliferation, metastasis, and apoptosis aversion (Yarden, Sliwkowski, 2001).

Raf-MEK-ERK Mitogen-Activated Protein Kinase Cascade

The most common EGFR signal transduction pathway is the Raf-MEK-ERK Mitogen-Activated Protein Kinase Cascade (Figure 2). This cascade contains a set of its own mutations throughout the steps of the pathway which are leading causes of cancer as well. Mammalian cascades involving Mitogen-Activated Protein Kinases (MAPK's) are part of a three step signaling process. The MAPK Kinase Kinase (MAPKKK) signals the MAP Kinase (MAPKK) which then signals the MAPK (Roberts, Der, 2007). In the EGFR MAPK cascade, active EGFR stimulates Raf, the MAPKKK, through association with adaptor protein Grb2 which recruits SOS-1 nucleotide exchange factors to the cell membrane. SOS-1 activates Ras proteins by exchanging the GDP bound to Ras for GTP. The activated Ras recruits Raf to the cell membrane and activates it through a complex process involving phosphorylation (Friday, Adjei, 2008). The Raf MAPKKK phosphorylates and activates MEK 1 and 2, the MAPKK's, which phosphorylate the MAPK's- Extracellular signal-regulated kinases (ERK 1 and 2). Activated ERK's go on to regulate numerous proteins and transcription factors, resulting in cell proliferation and survival. Although this pathway has previously been con-

sidered linear, in reality it involves many complex interactions with other pathways which regulate the different steps, mainly Raf Activation. Studies have shown that mutations in each step along the path of this complex pathway are present in a substantial percentage of cancers and other disorders. This complexity can be demonstrated with an example involving EGFR. EGFR is involved in the upstream and downstream regulation of the MAPK pathway. EGFR functions upstream by signaling the Ras protein in which case an EGFR mutation or over expression can cause hyperactivation of the MAPK pathway. Additionally, one of the products of ERK signaling is TGF- α and other EGFR ligands whose over expression can lead to hyperactivation of the pathway as well. In order to combat the numerous mutations which present themselves throughout this pathway, intense study and research is currently devoted to the development of Raf inhibitors, MEK inhibitors, Ras inhibitors, and EGFR inhibitors- our area of interest (Roberts, Der, 2007).

EGFR Inhibitors

As a result of the large proportion of EGFR mutations among Non Small Cell Lung Cancer, much effort has been devoted to the research and development of inhibitor drugs which combat the neoplasia resulting from the over-expression of the EGFR proteins. Novel target therapies in the midst of development can aim at the specific EGFR oncogene as opposed to affecting all the cells in the body with chemotherapy, which has proven itself relatively ineffective in treatment of lung cancer (Vealel, et. al. 1987). Various research groups are exploring distinct methods of inhibiting the hyperactivity of the EGFR pathway. This inhibition is relatively harmless because the EGFR pathway does not play an essential role in the bodies of healthy adults, its inhibition causing a rash or diarrhea at most (Fukuoka, et. al. 2003). Aside for the EGFR signal transduction pathway inhibitors discussed earlier, there are two forms of inhibitors of the EGF receptor itself currently in the form of marketable drugs. These two forms of inhibitors are monoclonal antibodies and tyrosine kinase inhibitors. Researchers are currently attempting to find the optimal combination of these two target therapies together with standard radiation and chemotherapy for each case of Lung Cancer. Monoclonal antibodies, such as Ce-

tuximab, are large molecules that act from outside of the cell. These antibodies inhibit EGFR activation by binding close enough to the ligand-binding site that it can block the ligand from binding to the receptor (Friday, Adjei, 2008).

Our inhibition drugs of interest are Tyrosine Kinase Inhibitors. Tyrosine kinase inhibitors are small molecules with the ability to move through the cell membrane in order to interact with the cytoplasmic EGFR domain. Tyrosine kinase inhibitors exhibit competitive inhibition, binding to the tyrosine residues of the EGF receptor, thereby blocking ATP from binding and activating the EGFR and its downstream effectors. This inhibition of the EGFR pathway has demonstrated clinical response and has prolonged life expectancy of EGFR mutation patients. It is believed that the tyrosine kinase inhibitors discontinue the translation of EGFR effectors which had induced cellular proliferation and metastasis, thereby reducing the excessive cell growth present in the malignancy (Massutí, 2003). Furthermore, it was proposed that tyrosine kinase inhibition induces apoptotic cell death by upregulation of Bim, a pro-apoptotic protein. Another strong possibility is that tyrosine kinase inhibitors induce cell death by inhibiting mTOR, an autophagy-inhibitor protein whose activity is normally increased by EGFR activity. Autophagy is the process of which the cellular organelles are swallowed up by lysosomal vesicles, but this process is normally regulated by the EGFR pathway. With the EGFR pathways halted, this inhibition is weakened and autophagy becomes more prevalent amongst the malignant cells causing the desired cell death (Markus, Alain, 2013).

Two TKI drugs currently on the market are Gefitinib/Iressa and Erlotinib/Tarceva. In the randomized, double blind, Phase II trial of Gefitinib, the results were promising. Included in the study were 210 advanced NSCLC patients who had been treated with chemotherapy once or twice and had received platinum treatment. They were chosen to receive a daily oral dose of either 250-mg or 500-mg Gefitinib. Tumor response rates were approximately 19% and the symptom improvement rate of evaluable patients was approximately 40%. Adverse effects were minimal in both cases consisting mainly of diarrhea and skin rashes (Fukuoka, et. al. 2003).

The Erlotinib results seem optimistic as well. The

international phase III Erlotinib trial was randomized, double blind, and placebo controlled with a 2:1 ratio of 150mg daily dose of Erlotinib to the placebo group. The placebo was considered ethical because further chemotherapy would not have benefitted these patients whose first or second bout of chemotherapy had failed. Response rate was around 9% in the group who received Erlotinib and less than 1% in the placebo group. Median survival was prolonged by two months (Shepherd, et. al. 2005).

EGFR Mutations

The control that EGFR contains over cell proliferation and metastasis makes it a key protein for oncogenic mutation. Hyperactivity of the EGFR pathway in any step of its complex network can lead to neoplasia. This can happen through mutation, over expression, or amplification of EGFR, its ligands, or a protein involved in the EGFR pathway.

In a study done in Japan (Kosaka, et. al. 2004), tumor samples were obtained from 277 randomly selected patients (Figure 3). Exons 18-21, the first four exons that code for the Tyrosine Kinase domain, were amplified and underwent molecular analysis, as these areas had been previously shown to contain the EGFR mutations in conducted studies. Mutations in this area of the tumor's EGFR gene were found in 111 patients (40%). There were 52 in-frame deletion mutations, 54 point mutations, and 5 duplication/insertion mutations. The deletions were all around the five amino acids ELREA from codons 746-750 of exon 19. Approximately half were simple deletions and half were deletions coupled with point mutations or insertions. A Thymine to Guanine exchange at the second nucleotide of codon 858 in exon 21 was found in 85% of the point mutations, leading to an exchange of Leucine for Arginine. In summary, 91% of the EGFR mutations occurred as deletions around E746-A750 or as L858R point mutations. These two forms of mutations have come up in numerous studies conducted in this area, proving to be strongly correlated with EGFR mutational Non-Small Cell Lung Cancer (NSCLC).

Clinical Features Correlated to EGFR Aberration

As numerous studies were conducted, specific patterns of clinical and pathological features were found to

be present in the lung cancer patients involved in the studies. Female gender, non-smoking history, Asian descent, and Adenocarcinoma were all found to be directly related to EGFR mutations in NSCLC. EGFR mutations are almost completely exclusive to adenocarcinomas in all conducted studies. In the Japanese study, only one out of 111 EGFR mutated patients had nonadenocarcinoma. In a similar study conducted in Italy (Marchetti, et. al.2005), EGFR mutations were found exclusively in the adenocarcinoma patients. Smoking history showed significantly high statistical correlation to EGFR mutations as well. Fifty nine percent of the patients in the Italian study with mutated EGFR proteins were nonsmokers and 41% were smokers or former smokers. The study in Japan takes this smoking correlation a step further after completing more advanced statistical analysis solely on the Adenocarcinoma patients. They divided the smokers into three categories depending on the length of the period of time spent smoking, and it was discovered that the higher the exposure to smoking, the lower the percentage of EGFR mutation was found in that subgroup of patients, refuting the hypothesis that smoking is related to NSCLC.

In terms of gender, in both the Japanese and Italian studies as well as numerous other studies conducted on this subject, the females were a significantly larger percentage of the NSCLC patients found with the EGFR mutation. However, because female patients are usually non-smokers as well as Adenocarcinoma type cancer patients, the Japanese study performed logistic regression analysis to determine if gender contributed independently to EGFR mutation. The result demonstrated that only Adenocarcinoma histology and smoking status contributed independently to EGFR mutation, while female gender was an outcome of the other features that were present amongst females in a higher percentage than males. Similar results were found in many other studies as well. Furthermore, an added observation was made on the small number of patients under the age of 50, menopause age in Japan. It was found that the EGFR mutation in these younger patients did not lean toward the female gender, further discounting theories of hormonal activity affecting the EGFR protein in females.

Japanese NSCLC patients were confirmed numer-

ous times for having a higher proportion of EGFR mutation than American NSCLC patients (Kosaka, et. al. 2004). However, this feature is likely a result of other commonly found features in Asian populations. There are a significantly lower percentage of NSCLC patients with a history of smoking in Japan than in America. In the Japanese study, 83% of the female and 10% of the male patients were non-smokers, which is typical of Japanese patients. The conductors of this study note that in a parallel American study, however, 15% of the female and 6% of the male patients were never-smokers. These results were characteristic of many studies which evaluated American versus Asian smoking history.

Discussion

There has been great success in the area of EGFR target therapy, as well as with target therapy in general as a cure for cancer. However, in order to continue in the path toward success, clearer biomarkers must be established which can help to narrow down the population to which the drug is originally administered. Currently, the percentage of response to EGFR TKI's is only around 10% (Fukuoka, et. al. 2003, Shepherd,et. al. 2005). The current goal of the ongoing research in this area is to hone in on this 10% of patients, using research and experimentation to solve the mystery of their success. Eventually, as the response to each target therapy is clearly understood, each patient will be given his or her optimal drug combination, and greater response rates will be seen with cancer patients worldwide.

An integral step toward this goal is the determination of objective patient features for each target therapy which will signal success (or failure) in the inhibition of the gene of interest. A standard, quantitative scoring system for assessment must be established to determine if the target therapy is, in actuality, blocking what it was intended to block and if that blockage elicits a positive clinical response. New technology is being developed to assist the research in this area such as cDNA Arrays which will allow the identification of genes regulated by anti-EGFR therapies. Protein Arrays could be used to explore the proteins involved in the EGFR pathways (Baselga, Arteaga, 2005).

Once clear signs of drug success have been estab-

lished, it is necessary to explore the correlation between clinical-pathological patient biomarkers and drug success (or failure). Research is being conducted to explore the patterns of clinical patient features which are more prevalent among patients who responded to the drug. The two forms of biomarkers being researched amongst NSCLC patients are genetics and clinical characteristics. The genetic sequence mutations amongst EGFR NSCLC patients are somewhat clear. The two main mutations are the E746-A750 deletion as well as the L858R point mutation. However, the clinical patient features commonly prevalent in EGFR mutation patients must be examined further. In order to accomplish this goal, there are those who believe that a “No tissue-No trial” rule should be enacted in which a patient whose molecules of interest are unclear in the tissue would be excluded from the trial. This way, only patients who will help research further its goal of establishing a connection between drug response and biomarkers would be granted the drug (Baselga, Arteaga, 2005).

In terms of clinical features, it seems that the two characteristics which independently correlate to EGFR mutation are adenocarcinoma type cancer and non-smoking history. Female gender prevalence in drug response is a likely outcome of its correlation to Adenocarcinoma and non-smoking history. The results of the logistic regression analysis in many studies and trials did not point to female gender with an independent correlation to drug response. Additionally, the analysis of the pre-menopause patients in the Japanese study points to the same conclusion; female gender does not independently correlate with EGFR inhibition response rate. In the Gefitinib trial, no pharmacokinetic differences were found between men and women (Fukuoka, et. al. 2003).

The prevalence of drug response in Asian populations does not hold its own when scrutinized with more complex methods of statistical analysis. Upon observing a higher response rate for Japanese patients in the Gefitinib trial, a pharmacokinetic analysis was conducted which did not reveal any differences between Japanese and non-Japanese patients. Additionally, after an extremely complex logistic analysis with multiple variables involved, Asian descent response rates were not considered statistically significant (Fukuoka, et. al. 2003).

However, the connection between EGFR mutation and the independent characteristics of adenocarcinoma and smoking history are pretty firmly established. Fukuoka (2003) postulates that the slow growth of adenocarcinomas may lend itself to successful drug response.

Conclusion

The true link between prevalent clinical traits and drug response rate can only be understood with a deeper understanding of the complex EGFR network of signal transduction pathways and cross talk found amongst them. This will be possible with the use of technology such as genetic and protein arrays as well as analysis of future studies. This understanding, coupled with the knowledge of clear biomarkers, will allow for target drug administration to specific populations and higher response rates in EGFR inhibitor drugs. Target cancer drugs will eventually be administered to the small percentage of the population who will likely respond to the drug, thereby efficiently creating a higher success rate of target therapy.

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